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Chromosomal Locations and Gonadal Dependence of Genes That Mediate Resistance to Ectromelia (Mousepox) Virus-Induced Mortality

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Four genetic loci were tested for linkage with loci that control genetic resistance to lethal ectromelia virus infection in mice. Three of the loci were selected because of concordance with genotypes assigned to recombinant inbred (RI) strains of mice derived from resistant C57BL/6 and susceptible DBA/2 (BXD) mice on the basis of their responses to challenge infection. Thirty-six of 167 male (C57BL/6 × DBA/2)F_1 × DBA/2 backcross (BC) mice died (22%), of which 27 (75%) were homozygous for DBA/2 alleles at *Hc* and *H-2D*. Twenty-eight percent of sham-castrated and 6% of sham-ovariectomized BC mice were susceptible to lethal mousepox, whereas 50% of gonadectomized mice were susceptible. There was no linkage evident between *Hc* or *H-2D* and loci that controlled resistance to lethal ectromelia virus infection in 44 castrated BC mice. Mortality among female mice of BXD RI strains with susceptible or intermediate male phenotypes was strongly correlated ($r = 0.834$) with male mortality. Gonadectomized C57BL/6 mice were as resistant as intact mice to lethal ectromelia virus infection. These results indicate that two gonad-dependent genes on chromosomes 2 and 17 and one gonad-independent gene control resistance to mousepox virus infection, that males and females share gonad-dependent genes, and that the gonad-independent gene is fully protective.

Host genes play an important role in determining the severity of many viral infections (3, 7). Mouse models of genetic resistance to viruses of various classes have shown that resistance is usually under polygenic control (7). This genetic complexity has stifled efforts to localize resistance loci and to determine their mechanisms of action. The development of recombinant inbred (RI) mice has provided a means for the dissection of genetically complex systems, although their primary purpose has been to map single gene traits (2). RI strains were used in this study to locate one of possibly three genes that control innate resistance to lethal mousepox virus infection.

Mousepox is an important disease of mice caused by ectromelia virus, an orthopoxvirus of the vaccinia virus subgroup (13). High mortality is common during epizootics in mouse colonies and is associated with virus dissemination to many organs, widespread necrosis, notably in the liver and spleen, and minimal inflammation (12, 19). Resistance to lethal infection is mouse strain dependent. Several inbred strains of mice, including C57BL and AKR, are resistant to the lethal effects of ectromelia virus infection, but most inbred strains are susceptible (6, 36, 40). Asymptomatic infections in resistant strains, like lethal infections, are usually generalized, but virus spreads more slowly, virus titers are lower, there is minimal necrosis, and hepatic and splenic lesions contain an abundance of mononuclear cell infiltrates (19, 29). The mechanisms that control the expression of genetic resistance are not known, but recent studies indicate (i) that T-cell-, natural killer cell-, and interferon-dependent host defenses must operate for resistance to be expressed, (ii) that specific T-cell precursors appear earlier in regional lymph nodes of resistant than susceptible mice, and (iii) that resistance mechanisms are expressed during early stages of infection (20, 29).

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Resistance to lethal mousepox virus infection in nonimmune mice is inherited as a dominant trait (8, 36, 40). Mendelian analyses of the susceptibility of segregant crosses between resistant C57BL/6 or C57BL/10 mice and various susceptible inbred strains suggest that one or more genes mediate resistance (8, 36, 40). In crosses involving multiple genes, there is a sex bias; more female mice than male mice are resistant (8, 40). Reciprocal backcrossing has shown that all resistance genes are autosomal in male and female mice, suggesting that the additional resistance of female mice is mediated through sex-limited expression of autosomal genes (8).

Studies by O'Neill and coworkers using C57BL/10 congenic mice have shown that the *H-2* haplotype modulates resistance to ectromelia virus, but the effects are relatively minor and appear late (30). Other loci, which appear to play a more important role, have not been mapped.

In this study, we localized two major resistance genes to regions on mouse chromosomes 2 and 17 near loci that encode C5 and H-2, respectively, and determined the role of the gonads in the expression of resistance.

MATERIALS AND METHODS

Mice. Male DBA/2NCr (D2/NCr) and C57BL/6NCr (B6/NCr) and female (B6/NCr × D2/NCr)F_1(F_1/NCr) mice were obtained from the Frederick Cancer Research Center, Frederick, Md. DBA/2J (D2/J), (C57BL/6J × D2/J)F_1(F_1/J), and C57BL/6J × D2/J (BXD) RI strains were obtained from Jackson Laboratory, Bar Harbor, Maine. Mice were specific pathogen free and were maintained under specific-pathogen-free conditions. First-backcross (BC) mice were produced from male D2 and female F_1 mice. BC mice were individually identified by ear punch and coat color.

Gonadectomy. Mice were anesthetized by immersion in ice chips for 3 min and castrated or ovariectomized at 4 to 7 days of age as described previously (35).

TABLE 1. Assigned parental genotypes based on two gene models of resistance to ectromelia virus-induced mortality and concordance with typed loci

Phenotype	BXD RI strains[a]	Assigned genotype		No. with concordant loci			
		Additive gene	Major gene	*Hc*	*Prp*	*Hbb*	*Lpl*
Resistant	2, 6, 8, 12, 15, 18, 19, 21, 23, 27, 28, 29, 30	*B*[b]	*B*	10/13	10/13	9/13	10/13
Intermediate	1, 11, 13, 14, 16, 22, 25, 31	*B* or *D*[c]	*D*	6/8[d]	4/8[d]	4/8[d]	6/8[d]
Susceptible	5, 9, 24, 32	*D*	*D*	4/4	4/4	4/4	4/4

[a] From reference 8.
[b] Genotype of C57BL/6.
[c] Genotype of DBA/2.
[d] Concordant for *D* of major gene model.

Blood samples. Mice were bled from the periorbital venous plexus by using heparinized microhematocrit capillary tubes. Samples were centrifuged, plasma, buffy coat, and packed erythrocytes were separated by scoring tubes with a diamond pen under magnification, and contents were expelled into microcentrifuge tubes placed on ice. Packed erythrocytes were expelled into tubes containing 40 μl of distilled water, buffy coats were expelled into 200 μl of RPMI 1640 with 5% fetal calf serum, and plasma was expelled into empty tubes. Retained erythrocytes were lysed in buffy coat samples by hypotonic shock. Buffy coat cells were then suspended in 200 μl of RPMI 1640 containing 5% fetal calf serum.

Assays for *Hc*, *H-2D*, *Hbb*, and *Es-1*. Hemolytic complement (*Hc*) was assayed in plasma samples as previously described (9). Briefly, 40 μl of plasma was added to microcentrifuge tubes containing 25 μl of a 2.5% suspension of washed sensitized sheep erythrocytes in Veronal buffer with calcium and magnesium chloride (pH 7.4) (modified Veronal buffer) and incubated in a 37°C water bath for 1 h. Sheep erythrocytes were sensitized with optimally diluted rabbit anti-sheep erythrocytes (Organon Teknika Corp., West Chester, Pa.). Cold modified Veronal buffer (500 μl) was added, samples were centrifuged, and A_{410} was read on a spectrophotometer. Zero and complete lysis controls in addition to plasma samples from D2 and F_1 mice were included with each run. Buffy coat cells were assayed for the presence or absence of *H-2D*^{b} by using a microcytotoxicity assay in flat-bottom 96-well plates (26). Buffy coat suspensions in 200 μl were divided into two wells, one of which received 5 μl of optimally diluted mouse monoclonal immunoglobulin G1 anti-mouse *H-2D*^{b} (Chemicon International, El Segundo, Calif.). After 15 min of incubation at room temperature, 50 μl of optimally diluted guinea pig complement was added to both wells, which were then incubated for 45 min at 37°C. To each well was added 100 μl 0.1% nigrosin in Hanks balanced salt solution, and the wells were then refrigerated for 30 min. Plates were read on an inverted microscope. Buffy coat cells from D2 and F_1 mice were included with each run. Cellulose acetate electrophoresis of mouse hemoglobin modified by cystamine was used to type hemoglobin beta-chain (*Hbb*) alleles (42). Serum esterase 1 (*Es-1*) alleles were determined in plasma samples by using cellulose acetate electrophoresis (32). Hemoglobin and plasma from D2 and F_1 mice were included with each run.

Virus. Stocks of the Moscow strain of ectromelia virus were prepared, titered, and stored as previously described (8). The titer of stock virus was 2×10^9 PFU/ml, as determined by inoculation of BS-C-1 cell culture monolayers (21).

Mouse inoculation and observation. Mice were lightly anesthetized with methoxyflurane (Pitman-Moore, Washington Crossing, N.J.) and were inoculated subcutaneously between the shoulders with 10^5 PFU of virus in 0.1 ml. This dose of virus and route of inoculation consistently caused greater than 95% mortality in D2 mice and less than 5% mortality in B6 and F_1 mice, as determined in multiple experiments and as previously reported (6, 8, 19, 20). Mice were examined daily for clinical signs; when deaths occurred, gross examinations were made to confirm that mousepox lesions (hepatic and splenic necrosis) were present. Over 95% of all BC and RI mice that died did so between 8 and 11 days postinoculation. Deaths were scored for 21 days. Four weeks after inoculation, surviving mice were killed with methoxyflurane and their serum was tested for antibody to vaccinia virus by an immunofluorescence assay (21). All BC mice were inoculated between 4 and 6 weeks of age unless otherwise noted. BXD RI strains were inoculated between 5 and 10 weeks of age.

Assignment of parental genotypes to BXD RI strains. In a preliminary study, we assigned phenotypes to 25 BXD RI strains on the basis of susceptibility of male mice to lethal mousepox virus infection; 13 strains were indistinguishable from B6 mice and were classified as resistant, 4 strains were indistinguishable from D2 mice and were classified as susceptible, and 8 strains had mortality rates between those of the parent strains and were therefore classified as intermediate (8). Because this strain distribution pattern (SDP) was not consistent with a single segregating gene, and because a previous study (40) of BC mice derived from the progenitors of the BXD RI strains suggested that two independently segregating genes might determine male susceptibility (female mortality was lower, indicating greater penetrance of or more resistance genes), we assumed that at least two genes determined the RI SDP. The observed SDP could have resulted from two genes with additive effects or from one major and one minor gene. As determined from the additive gene model, resistant strains had both resistance alleles, intermediate strains had either of the resistance alleles, and susceptible strains had neither resistance allele. On the basis of the major/minor gene model, resistant strains had the major resistance allele, intermediate strains had the minor resistance allele, and susceptible strains had neither. Parental genotypes were assigned to each strain on the basis of these assumptions (Table 1). With the additive gene model, only the 17 resistant and susceptible strains were assigned parental genotypes. With the major gene model, all 25 RI strains were assigned parental genotypes.

Statistics. Chi-square analysis was used to compare SDPs in RI strains, to test for linkage in BC mice, and to test the effects of gonadectomy on BC mice. Spearman's rank correlation was used to compare male and female mortality in

TABLE 2. Associations of *Hc* and *H-2D* genotypes with deaths from mousepox in segregating male (C57BL/6 × DBA/2)F_1 × DBA/2 mice

Substrain	Fraction of genotype dying (%)				
	$Hc^{0/0}$	$Hc^{0/1}$	$H\text{-}2D^{d/d}$	$H\text{-}2D^{d/b}$	Combined
Jax[a]	13/42 (31)	6/59 (10)[b]	14/50 (28)	5/51 (10)[c]	19/101 (19)
NCr[d]	14/33 (42)	3/33 (9)[e]	13/32 (41)	4/34 (12)[b]	17/66 (26)
Jax + NCr	27/75 (36)	9/92 (10)[f]	27/82 (33)	9/85 (11)[f]	36/167 (22)

[a] C57BL/6J and DBA/2J progenitors
[b] $P < 0.025$ versus homozygous genotype.
[c] $P < 0.05$ versus homozygous genotype.
[d] C57BL/6NCr and DBA/2NCr progenitors.
[e] $P < 0.01$ versus homozygous genotype.
[f] $P < 0.001$ versus homozygous genotype.

RI strains. The proportions of castrated and intact BC mice of specific genotypes were compared with a z test. Differences that yielded P values of less than 0.05 were considered significant, and those with P values of less than 0.01 were considered highly significant.

RESULTS

Selection of loci for linkage analysis. Parental genotypes were assigned to BXD RI strains of mice on the basis of their susceptibility to lethal mousepox virus infection and on the assumption that resistance was mediated by two independently assorting genes, one major and one minor or both additive (see Materials and Methods). These SDPs were compared with SDPs for 250 loci typed in BXD RI strains. There was significant concordance between SDPs for one or both genetic models and four loci, *Hc* on chromosome 2, *Prp* on chromosome 6, *Hbb* on chromosome 7, and *Lpl* on chromosome 8 (Table 1). Two of these loci, *Hc* and *Hbb*, could be typed by using peripheral blood. *Lpl* could not be typed from peripheral blood, but *Es-1*, a locus 7 centimorgans from *Lpl* (22), could be typed. Therefore, *Hc*, *Hbb*, and *Es-1* were selected for linkage testing along with *H-2D*. The latter was selected because of the previously assigned minor role of *H-2* haplotype in resistance to lethal mousepox virus infection, although concordance between *H-2* haplotype and the genetic models was not significant.

Linkage between resistance or susceptibility and *Hc* and *H-2D*. Linkage was tested between resistance or susceptibility to lethal mousepox virus infection and four loci in F_1 × D2 BC mice, using two sets of substrains as the progenitors. Only male BC mice were tested because they express fewer resistance genes than do female mice (8, 40) and because male mice were used to determine resistance phenotypes in BXD RI strains. B6/J and D2/J substrains were selected because they were the progenitors of the BXD RI strains. Males of this BC (BC/J) exhibit polygenic resistance to lethal mousepox virus infection (12% mortality [40]). Four loci were typed in 101 BC/J mice that were subsequently infected with ectromelia virus. Nineteen mice (19%) died. There was significant concordance between deaths and *Hc* and *H-2D* (Table 2) but not *Hbb* or *Es-1* (data not shown). Mice that were homozygous for Hc^0 or $H\text{-}2D^d$ died at about triple the number of mice that were heterozygous for each locus. The second substrains, B6/NCr and D2/NCr, were selected because male BC mice derived from them (BC/N) exhibited 50% mortality after infection (8), suggesting that fewer resistance loci were segregating than in BC/J mice. A total of 66 BC/N mice were treated identically to the BC/J mice. Seventeen mice (27%) died, below expectations for a single gene but similar to the death rate in BC/J. There were highly significant associations between deaths from mousepox and *Hc* or *H-2D* (Table 2), but not *Hbb* or *Es-1* (data not shown). More than four times as many mice that were homozygous for Hc^0 died than mice that were heterozygous at this locus, and more than three times as many mice that were homozygous for $H\text{-}2D^d$ died than mice that were heterozygous at this locus. Because mortality between the two experimental groups did not differ significantly, the results were combined. There was highly significant concordance between virus-induced deaths and both loci. When mice were divided into four groups on the basis of combined genotypes, 61% (22 of 36) of BC/J+N mice that died were homozygous for D2 alleles at *Hc* and *H-2D*, whereas only 25% would have been expected to be double homozygous were there no linkage. Twenty-two of 41 (54%) double-homozygous BC/J+N mice died, whereas 4 of 51 (8%) double-heterozygous mice died and 5 of 41 (12%) and 5 of 34 (15%) single-heterozygous mice died. These studies indicated that two resistance genes linked to *Hc* and *H-2D* mediated resistance and that inheritance of Hc^l or $H\text{-}2D^b$ from the resistant parent decreased the risk of dying by a factor of about 4 compared with mice that did not inherit either allele.

Effect of gonadectomy on resistance. BC/N mice were gonadectomized or sham gonadectomized at 4 to 7 days of age and infected with ectromelia virus at 8 to 9 weeks of age; deaths scored for 21 days to determine whether sex-determined differences in resistance to lethal mousepox virus infection depended on gonadal function. At the time of infection, there were no significant size differences between mice in gonadectomized and sham-operated groups. There were significantly more deaths among gonadectomized than among sham-operated groups of both sexes. Gonadectomized male (group $n = 50$) and female (group $n = 48$) mice died at the same rates, about 50%, and sham-operated females (group $n = 50$) had significantly lower mortality (6%) than sham-operated males (group $n = 50$, 28% mortality). These results indicated that differences in resistance between male and female BC mice could be eliminated by gonadectomy, that some of the segregating resistance genes in both sexes were under gonadal control, and that in the absence of gonads, male and female BC mice exhibit mortality consistent with resistance mediated by a single gonad-independent gene.

Effect of gonadectomy on linkage with *Hc* and *H-2D*. The effect of gonadectomy on linkage between resistance to mousepox and *Hc* and *H-2D* was examined in 44 male BC/J mice that were castrated at 4 to 7 days of age, typed for *Hc* and *H-2D*, and challenged with ectromelia virus at 5 to 6 weeks of age. The death rate did not differ significantly from that observed in castrated BC/N male mice. Unlike intact mice, there was no detectable linkage between susceptibility to lethal ectromelia virus infection and *Hc* or *H-2D*. Death rates for $Hc^{0/0}$ and $Hc^{0/1}$ were 10 of 21 and 8 of 21, respectively, and death rates for $H\text{-}2^{d/d}$ and $H\text{-}2^{d/b}$ were 10 of 19 and 7 of 25, respectively. These results suggested that resistance alleles linked to *Hc* and *H-2D* were not expressed or had reduced penetrance in the absence of gonadal influences and that this could account for the increased mortality in gonadectomized mice. To determine whether the increased mortality in castrated versus intact BC mice could be attributed to the loss of protection mediated by resistance alleles linked to *Hc* and *H-2*, mortality was compared between mice in this study and intact mice of the same substrain cross and age from the previous study. Although

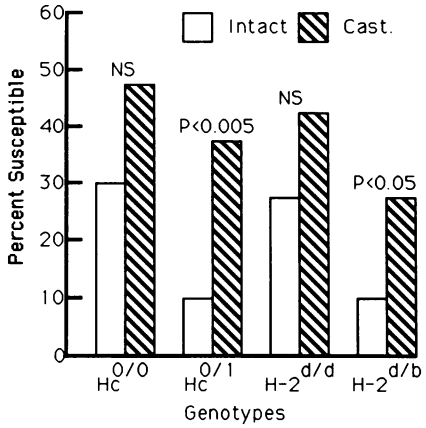

FIG. 1. Effects of castration on susceptibility to lethal mousepox virus infection in male BC mice of specific *Hc* and *H-2D* genotypes. Cast., Castrated; NS, not significant.

death rates were higher in castrated mice of all genotypes, they were only significantly higher in mice that inherited the $H\text{-}2D^b$-linked or the Hc^1-linked alleles (Fig. 1).

Evidence that males and females express the same resistance genes. Female mice of selected BXD RI strains were infected with ectromelia virus, and mortality was compared with that of male mice of the same strains. Females of the four susceptible strains and seven of the eight strains classified as intermediate were tested. In 8 of 11 cases, female mortality was less than male mortality; in 2 cases they were the same; and in 1 case female mortality was greater than male mortality (Table 3). There was a highly significant rank correlation between male and female mortality ($r = 0.834$, 9 df). These results indicated that female and male mice shared most resistance and susceptibility genes and that for most genotypes females were more resistant than males.

Effect of gonadectomy on resistant phenotype in C57BL/6 mice. Evidence from previous studies suggested that one major resistance gene protected gonadectomized BC mice and possibly $Hc^{0/0}$ $H\text{-}2D^{d/d}$ BC mice from lethal mousepox. The ability of this gene to protect mice in the absence of expression of gonad-dependent resistance loci was examined in gonadectomized male and female C57BL/6NCr mice. A total of 15 male and 15 female mice were gonadectomized and infected at 8 weeks of age. All survived infection.

TABLE 3. Ectromelia virus-induced mortality in males and females of BXD RI strains previously classified as intermediate or susceptible

BXD RI strain	Mortality (phenotype)[a]	
	Male	Female
1	3/8 (I)	1/8 (R)
5	8/8 (S)	8/8 (S)
9	7/8 (S)	4/8 (I)
13	4/8 (I)	0/8 (R)
14	3/8 (I)	1/8 (R)
16	6/8 (I)	4/8 (I)
22	2/8 (I)	0/8 (R)
24	8/8 (S)	4/7 (I)
25	5/8 (I)	8/8 (S)
31	4/6 (I)	1/8 (R)
32	8/8 (S)	7/7 (S)

[a] I, Intermediate; S, susceptible.

DISCUSSION

This study indicates that male B6 mice are protected from the lethal effects of mousepox by three unlinked genes or gene complexes, two of which are not expressed in castrated mice and are located on chromosomes 2 and 17. The resistance alleles of these genes appear to mediate protection independently, which would account for the 80% survival rate among male BC mice. Any of these genes may be identical to the locus described by Wallace and coworkers (40) and provisionally termed *Rmp-1* (resistance to mousepox 1). *Rmp-1* has been defined through studies of BC mice derived from B6 and susceptible A strain mice infected with the NIH-79 strain of ectromelia virus, but it has not been mapped (40). $Rmp\text{-}1^r$-mediated protection is dominant, affects males and females nearly equally, and is apparently the only polymorphous resistance gene expressed in that cross when mice are infected between 6 and 8 weeks of age (40). It is not known whether the expression of *Rmp-1* is gonad dependent. We propose that the gonad-dependent genes linked to *Hc* and *H-2D* be provisionally named *Rmp-2* and *Rmp-3*, respectively, with the r (resistance) alleles for both derived from B6 mice and the s (susceptible) alleles for both derived from D2 mice. This proposal is based on the assumption that *Rmp-1* was the gonad-independent gene which protected gonadectomized male and female BC and C57BL/6 mice equally or was not expressed in the crosses. Resistance mediated by the gonad-independent gene apparently develops in younger mice than that mediated by the gonad-dependent genes because resistance in B6 mice first appears between 14 and 21 days of age (20), prior to the synthesis of significant amounts of gonadal hormones (25).

The chromosome 2 and 17 loci mediated additional protection in intact BC mice, but it is not clear whether other gonad-dependent loci were also involved. Mortality was greater in castrated than in intact $Hc^{0/0}$ and $H\text{-}2^{d/d}$ BC mice, which presumably did not express the Hc^1- and the $H\text{-}2D^b$-linked resistance alleles. This result suggests that other loci might be involved, although the differences were not significant. We did not test for linkage between *Hc* and *H-2D* and resistance or susceptibility in female BC mice, but the strong correlation between male and female mortality in BXD RI strains showed that males and females shared most resistance loci. This finding raises the question of whether differential expression of shared gonad-dependent loci by males and females could account for the sex bias or whether more female mice were resistant by virtue of the expression of additional loci that were responsive only to ovarian products.

Genetic or hormonal sex factors are involved in resistance to other viral infections, but both differ from those revealed in this study. Genetic sex factors are controlled by alleles on the X chromosomes and are usually not influenced by gonadectomy (31, 44). Resistance to herpes simplex virus-induced hepatitis is an example of a genetic sex factor that controls the early interferon response (31, 44). Hormonal sex factors are stimulated by gonadal steroids and are therefore sensitive to gonadectomy (4, 5, 15, 34, 43). Resistance to coxsackievirus group B-induced myocarditis and resistance to encephalomyocarditis virus-induced mortality are controlled by hormonal sex factors that influence T-cell responses and early interferon responses (4, 5, 15, 34, 43). They are stimulated by the gonads of one sex only and are

expressed as a gender bias in the parental strains. The sex factors involved in resistance to mousepox virus infection behaved like hormonal sex factors in that they were sensitive to gonadectomy. Unlike the other examples, however, they were expressed through autosomal alleles, were stimulated by gonads of both sexes, and were not expressed as a gender bias in either parental strain but only in segregant progeny.

Studies by O'Neill and coworkers showed that *H-2* haplotypes have a relatively minor effect on susceptibility to lethal mousepox virus infection when they are congenic on a B10 background (30). The *H-2* haplotypes of B6 ($H\text{-}2^b$) and D2 ($H\text{-}2^d$) mice on the B10 background have indistinguishable effects on resistance. B10 mice, like B6 mice, express at least one non-major histocompatibility complex-linked major resistance gene (30). The major effects of the *H-2* haplotype on resistance to lethal mousepox virus infection in the present study may reflect their expression in the absence of other resistance alleles. This appears to be the case, since BC mice of both *H-2* haplotypes had similar mortality rates when they also carried the Hc^1-linked resistance allele, whereas the *H-2* haplotype had a major effect on mortality in $Hc^{0/0}$ BC mice. It is also possible that *H-2*-linked resistance is a function of heterozygosity at the *H-2* locus in BC mice and not of the haplotype per se. Heterozygosity at *H-2* confers higher endogenous natural killer cell activity than homozygosity (24), and natural killer cells are known to play a protective role in mousepox (20). A third possibility is that if *H-2*-linked resistance was mediated by loci outside of the *H-2* complex, then it was more likely to remain *H-2* associated in first-BC mice than in congenic mice that have undergone multiple backcrosses affording multiple opportunities for crossovers.

Localization of gonad-dependent resistance loci to centromeric segments of chromosomes 2 and 17 invites speculation as to whether *Rmp-2* and *Rmp-3* are typed loci with pleiotropic effects which include resistance to mousepox virus infection. On both chromosome segments, complement components are encoded, levels of which are influenced by sex steroids (27, 39, 41) and play an important role in determining the outcome of certain viral infections (18). The role of complement in resistance to ectromelia virus infection is unknown. D2 mice are genetically deficient in C5 relative to B6 mice as a result of allelic differences at *Hc* (28). Genetically or experimentally lowered serum C5 levels predispose mice to the lethal effects of several viruses (18). Castration lowers serum C5 concentration as a result of the withdrawal of testosterone (39). There is no indication, however, from other inbred strains of mice that the *Hc* locus is *Rmp-2*. Several $Hc^{1/1}$ strains of mice (BALB/c and C3H) are highly susceptible to mousepox virus infection (8, 9). In addition, ovariectomy leads to an elevation in C5 levels (39) and would therefore be expected to increase resistance to ectromelia virus among female BC mice if the *Hc* locus is *Rmp-2*. There are multiple loci within or near the *H-2* complex that regulate complement proteins (23, 33). The products of some of these loci are sex steroid responsive (16, 41), and some show considerable variation between strains of mice (1, 41). D2 mice are deficient in C3 relative to B10 mice, but direct comparisons with B6 mice have not been made (14). The C3 structural locus is approximately 10 centimorgans telomeric to *H-2* (23). Levels of serum C3 are androgen but not estrogen responsive (39). Class III loci within *H-2* encode the C2 and C4 structural genes and a C3 regulatory sequence (10, 33). Since C2 and C4 serum levels are higher in $H\text{-}2^d$ than in $H\text{-}2^b$ mice (16, 17), relative deficiencies of C2 or C4 are not the basis of susceptibility to mousepox. Therefore, *Rmp-2* and *Rmp-3* do not appear to represent pleiotropic effects of loci that regulate complement components.

The possibility that *Rmp-3* is a class I or II gene of *H-2* must be considered in light of the importance of these genes in immune responses to viral infections. Sex steroids exert quantitative effects on immune responsiveness (11, 38), and autoimmune diseases linked to class I or II genes have sex biases (37). The strict dependence of *Rmp-3* upon gonadal function for expression, however, is not typical for class I or II genes.

Much has been learned about viral genes that regulate viral entry and replication in the mammalian host, but little is known about host genes that counteract or facilitate this process. The localization of two mouse genes that influence resistance to ectromelia virus and their dependence on gonadal function should facilitate the search for corresponding phenotypic mechanisms.

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